ProteinExt® Mammalian Membrane Protein Extraction Kit

Cat. No. DE301

Storage: *ProteinSafe*™ Protease Inhibitor Cocktail, EDTA-free (100×) at -20°C for one year, others at 2-8°C for one year

Description

ProteinExt® Mammalian Membrane Extraction Kit provides a simple and efficient method to extract membrane proteins from mammalian cells and tissues. Native proteins can be obtained within 70 minutes without ultracentrifugation. Up to 90% efficiency for membrane proteins have at least 1-2 transmembrane domains. The extracted protein is suitable for a variety of downstream applications, including SDS-PAGE, Western Blot, ELISA, and enzyme-activity assays.

Kit Contents

Component	DE301-01 (50 rxns)
Membrane Protein Extraction Buffer I (MPEB I)	50 ml
Membrane Protein Extraction Buffer II (MPEB II)	7.5 ml
Membrane Protein Extraction Buffer III (MPEB III)	15 ml
ProteinSafe™ Protease Inhibitor Cocktail, EDTA-free (100×)	1 ml

Procedures

a. Cultured Cells

1. Harvest 0.5-1×10^7 cells and wash the cells with 1 ml of pre-chilled PBS. Centrifuge at 1,000×g for 3 minutes. Discard the supernatant. Repeat the wash once.
2. Add 750 µl of MPEB I to cell pellet. Mix thoroughly by vortexing for 15 seconds. Incubate on ice for 10 minutes with vortexing at every 2 minutes.
3. Centrifuge at 16,000×g, 2-8°C for 15 minutes.
4. Gently transfer the supernatant (cytoplasmic protein) to a new 1.5 ml microcentrifuge tube. The isolated cytoplasmic proteins can be used for downstream applications or stored at -80°C.
5. Add 150 µl of MPEB II to the pellet and resuspend the pellet by vortexing for 15 seconds. Incubate on ice for 30 minutes and briefly vortex at every 5 minutes.
6. Add 300 µl of MPEB III to the pellet and vortexing for 5 seconds.
7. Centrifuge at 16,000×g, 2-8°C for 15 minutes.
8. Gently collect the supernatant (membrane proteins). The isolated membrane proteins can be used for downstream applications or stored at -80°C.

b. Tissues

1. Wash 20-60 mg of tissues with 2 ml of pre-chilled PBS and vortex briefly, gently discard the supernatant.
2. Add 1 ml of PBS. Cut 20-60 mg of tissues into small pieces. Centrifuge at 500×g for 3 minutes, gently discard the supernatant
3. Add 1 ml of MPEB I to the tissues and vortex thoroughly. Transfer the suspension to a pre-chilled glass homogenizer and homogenize the tissue normally by 6-10 strokes.
4. Incubate on ice for 10 minutes with vortexing at every 2 minutes.
5. Following steps are the same as the steps 3-8 described in “Cultured Cells” section.

Notes

• Prior to use, Proteinase Inhibitor Cocktail and PMSF (not provided in the kit) should be added into MPEB I and II and III.
• All steps should be carried out on ice or at 2-8°C.
• If protein quantification is needed, we suggest to use BCA method (*Easy* II Protein Quantitative Kit (BCA), Cat. No DQ111).

Service telephone +86-10-57815020
Service email complaints@transgen.com